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Preparation of hydrogel by radiation for the healing of diabetic ulcer

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HIGHLIGHTS

- The CMC hydrogel involving chestnut honey was prepared by gamma radiation.
- The physical properties such as swelling percent and gelation were examined.
- The chestnut hydrogel dressings displayed a prominent healing effect for diabetic ulcers.

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ABSTRACT

Honey has been used in wound care for thousands of years. The major advantage of honey in wound care is the high osmotic activity, which accelerates the debridement of necrotic tissue and procures an antibacterial effect. It has been reported that the ancient Greeks and Romans used honey as a topical antiseptic for sores and skin ulcers. The aims of this study were to evaluate the antibacterial activities and the healing effect for diabetic ulcers from carboxymethyl cellulose (CMC) hydrogel involving honey.

Carboxymethyl cellulose (CMC) and honey were dissolved in deionized (DI) water, and then irradiated by a gamma-ray to make a honey hydrogel dressing. The physical properties such as gelation and swelling were examined to evaluate the hydrogel for wound dressing. The antibacterial activities were investigated in detail against the *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) strains. Antibacterial tests indicated that honey hydrogel dressings have a good antibacterial activity. Female db/db mice (weight between 18 and 24 g, aged 5 weeks) were given an in vivo wound healing assessment. The wound dressing was changed every 2 days, and the rate of wound contraction and microscopic observations were observed.

The honey hydrogel dressings displayed a prominent healing effect for diabetic ulcers.

1. Introduction

According to the Center for Disease Control, 7.8% of the United States population has diabetes with an estimated lifetime risk of developing a foot ulcer of 25%. Over 14–24% of these patients will have progressive disease that necessitates amputation. Of the 116 billion dollars attributed to the medical care of diabetic patients, an estimated one-third of these costs are related to the treatment of diabetic foot ulcers (Blumberg et al., 2012).

In individuals with diabetes mellitus, wounds remain in a chronic inflammatory state and fail to heal in a timely and orderly manner. Continual influx of inflammatory cells and sustained production of their inflammatory mediators cause imbalances in wound proteases and their inhibitors, preventing ECM synthesis and remodeling that are essential for normal wound healing. Diabetes impairs the functions of neutrophils and macrophages, including cell adherence, chemotaxis, phagocytosis, and cytokine production and secretion.

The concept of moist wound healing has been examined and gradually accepted by wound care clinicians, and has led to the development of hydrogel dressing that supports a moist wound environment. Hydrogels are able to donate moisture to dehydrated tissue, absorb some moisture from an exudating wound, and work as debriding agents in the management of a variety of wounds (De la Brassinne and Thirion, 2006; Lim et al., 2009).

Hydrogels can be described physically as a three-dimensional network of hydrophilic polymers. Depending on the type of hydrogel, they contain a varying percentage of water. Despite their high intrinsic water content, hydrogels are capable of absorbing additional liquid owing to the presence of hydrophilic residues which allow hydrogels to swell extensively without changing the gelatinous structure.

Hydrogels can be prepared by various methods including chemical and radiation induced crosslinking. The radiation

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technique has several advantages such as easy process control, the possibility of joining hydrogel formation and sterilization in one technological step, no necessity to add any initiators, and a crosslinker (Rosiak and Yoshii, 1999; Lee et al., 2005). Also, carboxymethyl cellulose (CMC) hydrogels not only have a unique property of absorption and retention of a significant amount of water, but also are friendly to the environment due to their nontoxicity, degradability and good biological compatibility (Fei et al., 1999; Wach et al., 2004; Lee et al., 2009; Park et al., 2012a, 2012b).

It was known that propolis honey has antibacterial activity in vitro, and clinical case studies have shown that application of honey to severely infected cutaneous wounds is capable of clearing infection from the wound and improving healing (Tshukudu et al., 2010; Sforcin and Bankova, 2011).

In this study, when honey was included in the hydrogel, the feasibility of using this hydrogel for the ulcer wound was investigated. The physical properties such as swelling percent, gel percent and antibacterial activity were examined. The in vivo performance of this hydrogel for wound healing was evaluated histologically using diabetic rat models.

2. Experimental

2.1. Materials

Carboxymethyl cellulose (M.W. 250,000, DS 1.2%) was purchased from Sigma-Aldrich Co., Ltd. (ST. Louis, USA). Propolis honey was obtained from Seoul Propolis Co., Ltd. (Daejon, Korea). They were used without further purification. Distilled water was used as a solvent in all experiments.

2.2. Preparation of hydrogels for ulcers

In this experiment, carboxymethyl cellulose (CMC) powder (20 wt%) and honeys (0, 5, 15 and 20 wt%) were dissolved in purified water by using a planetary centrifugal mixer (Thunky company, Japan), and then irradiated by a gamma-ray to make a honey hydrogel dressing. The mixtures were poured into a square dish (40 × 20 × 2 mm), where they were irradiated by a gamma-ray at a dose rate 10 kGy/h to make the honey-hydrogel dressings.

Next, the CMC hydrogels were crushed into a paste state by using a T25 digital ULTRA-TURRAX high-performance disperser (IKA, Germany).

2.3. Determination of gelation

The gel fraction (*Gel*%) was estimated by measuring the insoluble parts after washing them in distilled water. The hydrogels were first dried at 75 °C for 48 h until they reached constant weight, and the sole parts were then extracted by immersing the dried gels at 50 °C for 72 h. The remaining gels were kept in a 75 °C oven for 48 h to completely dry the samples. *Gel*% can be calculated by the following equation:

$$Gel(\%) = \frac{w_d}{w_i} \times 100 \tag{1}$$

where w_d is the weight of the oven-dried gel after extraction, and w_i is the initial weight of the dried gel before extraction (Park et al., 2012a, 2012b).

2.4. Determination of swelling

The degree of swelling can be described as the water absorptivity of the hydrogels. The hydrogels were immersed in distilled water for different times at room temperature until an equilibrium state of swelling was reached. After the excessive surface fluids were removed with filler paper, the weight of the swollen gels was measured at various time intervals. The procedure was repeated until there was no further weight increase. The degree of swelling (*Sw*) can be calculated by the following equation:

$$Sw(\%) = \frac{(W_s - W_i)}{W_i} \times 100 \tag{2}$$

where w_s is the weight of the swollen gels at various time intervals and w_i is the initial weight of the dried gel.

2.5. Antibacterial test

The antibacterial effect of hydrogels involving honey was examined on agar plates inoculated with *Escherichia coli* (*E. coli*) *and Staphylococcus aureus* (*S. aureus*) by using the disk diffusion test. The *S. aureus*/*E. coli* bacteria contained tryptic soy agar (TSA) plates were prepared by evenly smearing with100 μL freshly grown bacterial inoculums (10^6 cell/mL) and incubated at 37 °C in oven for 3 h. Then the plates were supplemented with the CMC–Na hydrogels stabilized Ag NPs samples of cylinder shapes (diameter: 8 mm and height: 10 mm) and incubated at 37 °C for 24 h, the clear ratios were obtained by measuring the inhibition zones and calculated as follows:

$$\text{Clear ratio } (\%) = \frac{(d_i^2 - d_0^2)}{d_0^2} \times 100 \tag{3}$$

where d_0 is the diameter of samples (8 mm), d_i is the diameter of clear zone after 24 h.

2.6. Animal test

Three experimental groups, namely, (a) non-treated control wound, (b) CMC hydrogel treated wound, and (c) propolis honey/CMC hydrogel, were tested for wound healing.

The female db/db mice (C57BLKS/J Iar−+Lepr^{db}/+Lepr^{db}, weight between 18 and 24 g, aged 5 weeks) were used for in vivo wound healing assessment. The skin of the mouse was shaved and disinfected using 70% ethanol. Two full thickness skin wound prepared by skin compressor for 24 h on the dorsum of each mouse's body after acclimatization for one week. The wound dressing was changed every 2 days, and the rate of wound contraction and microscopic observations were assessed for two weeks. Real-time photograph observations were followed and the healing rates of the wound area contractions were calculated. Three experimental groups for ulcer

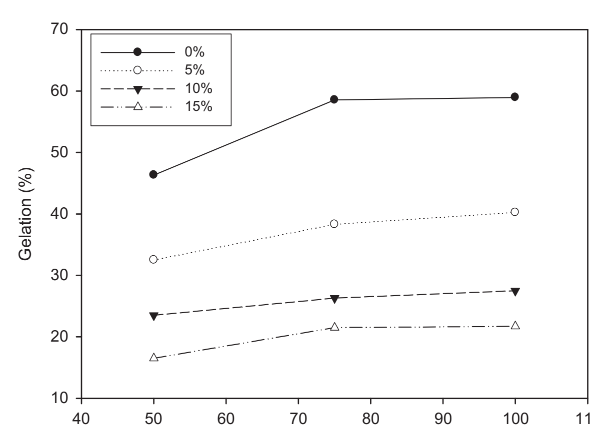

Fig. 1. Gel content of CMC/propolis honey hydrogels.

care were tested five times respectively. The wound size (W) of the db/db mice were calculated by using the following equation:

$$W(\%) = \frac{S_t}{S_0} \times 100 \quad (4)$$

where S_t is the wound area of mouse at time intervals, S_0 is the wound area of mouse at the initial time.

Also, the hydrogels were sterilized by a gamma-ray with an absorbed dose of 25 kGy before using them for animal test.

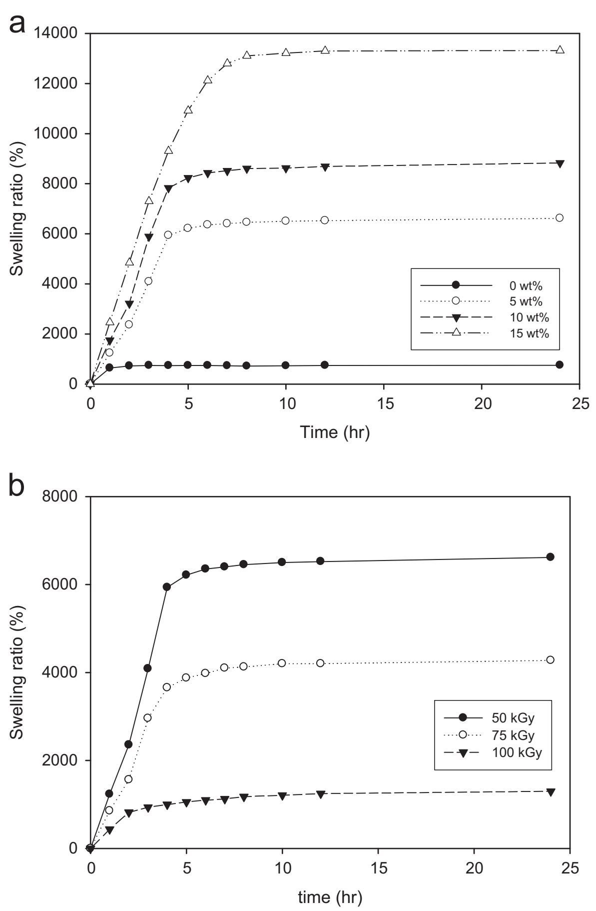

Fig. 2. Degree of the swelling of hydrogels involving propolis honey; (a) radiation dose is 50 kGy and (b) the content of honey is 5 wt%.

3. Results and discussion

3.1. Gel percent and swelling behavior

Crosslinking by radiation transforms a linear polymer into a three-dimensional molecule, resulting in a significant increase in the molecular mass, a lower solubility of the organic solvents, and improved mechanical properties. CMC was easily crosslinked in their homogeneous mixture with water by gamma radiation.

Fig. 1 shows the gelation behavior of the hydrogels involving honey as a function of irradiation dose. The gel content increased as the irradiation dose increased, while the gel content decreased as the concentration of honey increased. The decrease in gelation of the hydrogels by the addition of honey can be attributable to work as a plasticizer of honey in the hydrogels. Honey is a natural supersaturated sugar solution with water, fructose, glucose, maltose, sucrose and other types of carbohydrates. These components are not crosslinked by radiation, but work as a plasticizer.

Fig. 2 shows the swelling behavior of the CMC hydrogels involving honey. The degree of swelling containing honey was in the range of 300–13,000%. The degree of swelling of hydrogels containing honey decreased as the concentration of honey increased. It was observed that the polymer swelling decreased with an increase in irradiation dose. It is attributable that the three-dimension network in water increases with the irradiation dose, resulting in a restriction of mobility, which leads to a decrement in the hydrogel swelling. According to Figs. 1 and 2,

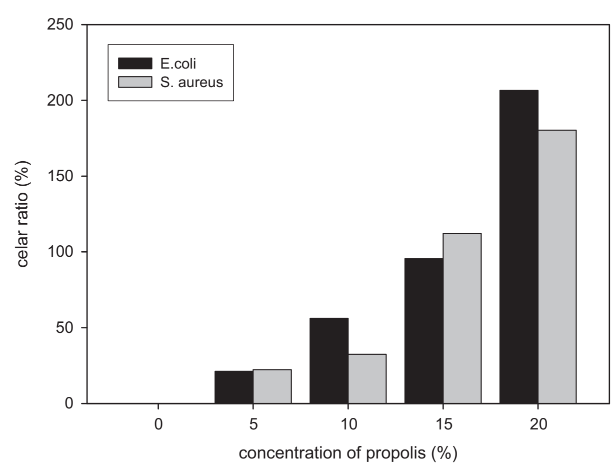

Fig. 4. Clear ratio of propolis honey/CMC hydrogel containing different honey contents against *E. coli* and *S. aureus*; radiation dose of 50 kGy.

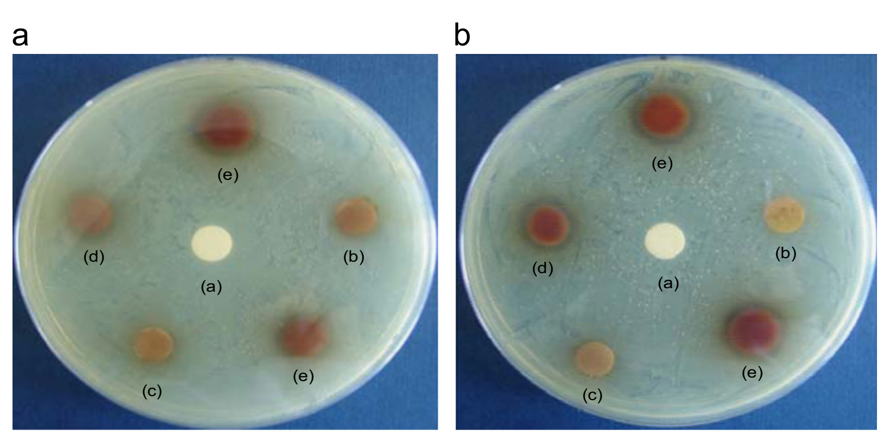

Fig. 3. Comparison of clear zones of (A) *E. coli* and (B) *S. aureus* in propolis honey/CMC hydrogel containing different honey contents: (a) 0 wt%, (b) 5 wt%, (c) 10 wt%, (d) 15 wt%, and (e) 20 wt%; radiation dose of 50 kGy.

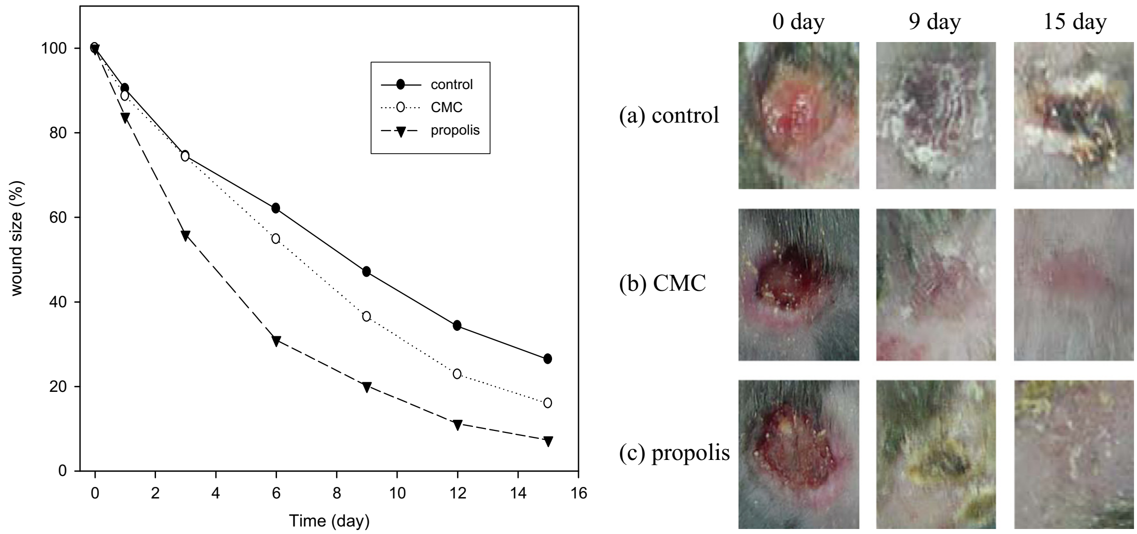

Fig. 5. Changes of wound covered with non-treated control wound (control), CMC hydrogel treated wound (CMC), and propolis honey/CMC hydrogel (propolis; the content of propolis is 20 wt%).

the percentage of swelling is inversely proportional to the gel percentage of the polymer chains.

3.2. Antibacterial effect

In this work, the antibacterial effect of propolis-CMC's against *S. aureus* and *E. coli* strains has been investigated. Inhibition zones around a honey-incorporated hydrogel were measured for a 24 h incubation period at 37 °C. Figs. 3 and 4 show the growth inhibition zones and clear ratio of propolis honey/CMC hydrogel containing different honey contents. The results show that propolis-honey based hydrogels have an effect on inhibiting and killing the two bacteria. Also, the antibacterial activity of hydrogels containing honey increased with increasing the concentration of honey. It is known that its property is attributable to the production of hydrogen peroxide, which paradoxically increases in concentration the more it is diluted. This is thought to be due to an enzyme present in honey that produces hydrogen peroxide when diluted.

Although many types of honey are available, the most commonly used medical dressings come from Manuka honey, which has been reported to have the best minimal inhibitory concentration to treat infections. Chesnut honey used in these experiments was shown to be effective against resistant strains of bacteria.

3.3. Healing effect of hydrogels

Honey impregnated dressings have been described in ancient texts for their use in various wounds, whether infected or not, to help promote healing with no adverse effects. Their utilization by the medical profession has become more prominent over the last few decades for a variety of indications.

The female db/db mice were used for in vivo wound healing assessment. Fig. 4 shows the wound image and recovery ratio of the ulcer. Three experimental groups, namely, (a) non-treated control wound (control), (b) CMC hydrogel treated wound (CMC), and (c) propolis honey/CMC hydrogel (propolis), were tested for wound healing. As shown in Fig. 5, the amount of wound exudates were observed in all groups until 9 days. In the case of the control group, the area of the wound was more spacious than other groups because the regeneration of wound was not progressed as much. Other groups, the CMC hydrogel and the propolis honey/CMC hydrogel were progressed at a similar ratio of the epithelial cell regeneration. Specially, propolis honey/CMC hydrogel hastened the healing rate by accelerating wound contraction compared with wounds treated only with CMC hydrogel.

4. Conclusion

CMC hydrogel involving propolis honey was prepared by gamma radiation for application to ulcer wounds. The hydrogel has an antibacterial effect against *S. aureus* and *E. coli*. The results from animal testing using diabetic rat models indicate that the CMC hydrogel involving propolis hastened the healing rate by accelerating the wound contraction compared with only CMC hydrogel. It was found that the hydrogel with propolis honey can be used as an effective wound dressing for ulcer wounds.

Acknowledgments

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